薬物による不飽和脂肪酸生合成制御の試み

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はしがき

食品中の成分が生体に対して多彩な生理作用を有し、その機能調節を行うことが、近年の研究から明らかになってきた。中でも脂肪酸は、単なるエネルギーの貯蔵ではなく、個々の脂肪酸によってその生理作用が異なっている。アラキドン酸、エイコサペンタエン酸、ドコサヘキサエン酸などはその生理作用が広く知られている。一方、オレイン酸はこれまで生理機能への積極的な関与が証明されていなかったが、エネルギー代謝の調節や、神経・免疫系などできわめて重要な働きをすることが次第に明らかとなってきた。

本研究代表者らはこれまでに、抗高脂血症薬であるクロフィブラートが肝臓においてΔ９不飽和化酵素を誘導することを明らかにしてきた。本研究では、この薬物が、ホルモンであるインスリンやデヒドロエピアンドロステロンと、あるいは、グルコースやフルクトースなどの糖類とどのようなクロストークをして、オレイン酸の生合成を調節しているかについて明らかにするとともに、一連のオレイン酸合成酵素がそれぞれどのように調節されるかを解明することを試みた。本研究の結果から、クロフィブラートなどの薬物は脂肪酸代謝を変動させるが、生理状態によってその影響が異なること、オレイン酸合成に関与する酵素は多様な調節を受けることなどが明らかとなった。これらの知見は、オレイン酸合成には多彩な調節機構があり、これをうまく制御すれば、薬物によってエネルギー代謝などの種々の生理機能を制御できる可能性を示すものである。本研究に対する文部省からの科学研究補助金の交付に対して深く感謝する次第である。

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研究課題

薬物による不飽和脂肪酸生合成制御の試み
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研究組織

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研究発表

（１）学会誌等

1. Mizuguchi, H., Kudo, N. and Kawashima, Y., Metabolic alterations by clofibric acid in the formation of molecular species of phosphatidylcholine in the liver of rats. Biochem. Pharmacol.., 62: 853-861 (2001)

2. Imai, K., Kudo, N., Koyama, M., Shirahata, A. and kawashima, Y., Effects of dehydroepiandrosterone on oleic acid formation in the liver of rats, mice and guinea pigs. Jpn. J. Pharmacol. 86: 437-447 (2001)

3. Imai, K., Kudo, N., Koyama, M. and Kawashima, Y., Effects of dehydroepiandrosterone on oleic acid accumulation in rat liver. Biochem. Pharmacol., 65: 1583-1591 (2003)

4. Kudo, N., Toyama, T., Mitsumoto, A. and Kawashima, Y., Regulation by carbohydrate and clofibric acid of palmitoyl-CoA chain elongation in the liver of rats. Lipids, in press.

（２）口頭発表

1. 外山智章、藤兼裕子、工藤なをみ、川嶋洋一
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日本薬学会第121年会　平成13年3月

2. 今井浩一、工藤なをみ、川嶋洋一、小山又二郎
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フォーラム2001：衛生薬学・環境トキシコロジー　平成13年10月

4. 外山智章、根岸裕一、工藤なをみ、川嶋洋一
糖による脂肪酸伸長酵素と脂肪酸不飽和化酵素誘導に対するクロフィブリンの影響
日本薬学会第 122 年会　平成 14 年 3 月

5. 工藤なをみ、今井浩一、川嶋洋一、小山又二郎
デヒドロエピアンドロステロンによる脂肪酸合成･分解への影響
日本薬学会第 122 年会　平成 14 年 3 月

6. 外山智章、田代美由樹、工藤なをみ、川嶋洋一
糖による脂肪酸伸長酵素と脂肪酸不飽和化酵素誘導に対するクロフィブリンの影響
日本薬学会第 123 年会　平成 15 年 3 月

7. 今井浩一、工藤なをみ、小山又二郎、川嶋洋一
糖尿病モデルラットにおける肝オレイン酸の合成活性とデヒドロエピアンドロステロンの影響
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研究成果

Regulation by Carbohydrate and Clofibric acid of Palmitoyl-CoA Chain Elongation in the Liver of Rats

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Running Title: REGULATION OF PALMITOYL-COA CHAIN ELONGATION BY CARBOHYDRATE

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Abbreviations:

The fatty acids are designated by the number of carbon atoms and double bonds;

16:0, palmitic acid;

16:1(n-7), palmitoleic acid;

18:0, stearic acid;

18:1(n-9), oleic acid;

18:1(n-7), vaccenic acid.

1-acly-GPC, 1-aclyglycerophosphocholine;

clofibric acid, 2-(*p*-chlorophenoxy)-2-methylpropionic acid;

PCE, palmitoyl-CoA chain elongation;

POCE, palmitoleoyl-CoA chain elongaiton;

PPARα, peroxisome proliferator-activated receptor α ;

SCD, stearoyl-CoA desaturase.

ABSTRACT

Regulation of palmitoyl-CoA chain elongation (PCE) and its contribution to oleic acid formation was investigated in rat liver in comparison with stearoyl-CoA desaturase (SCD). Hepatic PCE activity was induced by the administration of 20% wt/vol glucose or fructose for drinking in normal rats. In streptozotocin-induced diabetic rats, the activities of both PCE and SCD were suppressed and fructose, but not glucose feeding caused an increase in the activity of both enzymes. Treatment of normal rats with clofibric acid in combination with carbohydrate further increased PCE but not SCD activity. Fatty acid analysis of hepatic lipids revealed that oleic acid (18:1(n-9)) proportion was increased upon carbohydrate administration or clofibric acid. The treatment of rats with clofibric acid in combination with carbohydrate greatly increased 18:1(n-9) proportion. Significant correlation was observed between PCE activity and hepatic proportion of 18:1(n-9) (r^2=0.874, P<0.01), while the relationship between SCD activity and 18:1(n-9) proportion was not significant (r^2=0.552, P>0.05). Taken together, carbohydrate induces PCE activity as well as SCD to increase hepatic 18:1 content in rat liver, and the increased PCE activity seems to be responsible for the further increase in 18:1(n-9) when administered with carbohydrate in combination with clofibric acid.

INTRODUCTION

Biosynthesis of fatty acids plays an important role in both storing energy and providing the components of biomembranes in mammalian liver. Major parts of fatty acids are C16-C18 saturated and monounsaturated fatty acids that are initially synthesized *de novo* by fatty acid synthase using acetyl-CoA and malonyl-CoA. However, the end product of fatty acid synthase usually palmitic acid (16:0), and further desaturation and chain elongation are required to synthesize major C16-18 fatty acids such as stearic acid (18:0), palmitoleic acid (16:1(n-7)), oleic acid (18:1(n-9)) and vaccenic acid (18:1(n-7)) (1, 2). Stearoyl-CoA desaturase (SCD) was identified as an enzyme responsible for desaturation of both 16:0 and 18:0 to form 16:1(n-7) and 18:1(n-9), respectively in 1986 (3). It is known that SCD activity is greatly enhanced in response to feeding a fat-free diet, re-feeding after fasting and insulin, whereas it is suppressed by feeding polyunsaturated fatty acid or starvation (4-6). Recent progress has revealed molecular mechanisms responsible for the regulation of SCD (7-9). By contrast to SCD, information on fatty acid chain elongation has been limited although several lines of indirect evidence have suggested that endoplasmic reticulum contains at least three different enzymes catalyzing elongation of fatty acids (1, 10-13). Among them, palmitoyl-CoA chain elongation (PCE) that catalyzes stearic acid (18:0) formation from palmitic acid (16:0) was regulated by re-feeding after fasting, insulin and PUFA (10, 11, 14-16). In addition, various xenobiotics such as clofibric acid and diethylhexylphthalate induced the activities of both SCD and PCE (10, 11, 17, 18) although the mechanism of the induction has not been clarified yet. These observations suggest PCE is regulated by the mechanism similar to SCD. At the same time, it raised a question of how these enzymes individually contribute to 18:1(n-9) formation. To

answer the question, in the present work, we studied whether administration of carbohydrate increases PCE activity in rat liver in combination with clofibric acid and relative contribution of PCE and SCD to 18:1(n-9) formation. Physiological significance of PCE in 18:1(n-9) formation in the liver is discussed.

MATERIALS AND METHODS

Materials Stearoyl-CoA, palmitoyl-CoA, malonyl-CoA, 2-(*p*-chlorophenoxy)-2-methylpropionic acid (clofibric acid) and BSA were purchased from Sigma (St. Louis, MO). Triheptadecanoin and methyheptadecanoate were from Nu-Chek Prep Inc. (Elysian, MN); 1-acylglycerophosphocholine (1-acyl-GPC, from egg phosphatidylcholine) were from Avanti Polar Lipids, Inc. (Alabaster, AL); NADH, NAD and CoA were from Oriental Yeast Co. (Tokyo, Japan); [2-^{14}C]malonyl-CoA was from Moravek Biochemicals Inc. (Brea, CA); [1-^{14}C]16:0 was from American Radiolabeled Chemicals Inc. (St. Louis, MO); horse radish peroxidase was from Boeringher-Mannheim (Germany). All other chemicals used were of analytical grade.

Animals Male Wistar rats aged 5 weeks were purchased from SLC (Hamamatsu, Japan). After acclimatization for 1 week, some rats were given drinking water containing 20% wt/vol glucose or fructose for 2-7 days. In a separate experiment, rats were given drinking water containing 20% wt/vol glucose or fructose for 4 days. During the administration with glucose or fructose, half of each group was subcutaneously injected with clofibric acid at a dose of 100 mg/kg body weight twice a day for 4 days. Male Wistar rats of 4 weeks old were intravenously injected with streptozotocin dissolved in citrate buffer (pH 4.5) at a dose of 60 mg/kg body weight. Three weeks after streptozotocin injection, these rats were fed a fat-free diet (modified AIN93M of which carbohydrate and oil were substituted by glucose or fructose) for 4 days. Blood samples were collected under light ether anesthesia and then rats were killed by decapitation. Livers were quickly excised, perfused with ice-cold 0.9% wt/vol NaCl and

rinsed in 0.25 M sucrose/1 mM EDTA/10 mM Tris-HCl (pH 7.4). Then livers were homogenized with 4 volumes of the same solution in a Potter glass-Teflon homogenizer. An aliquot of the homogenates was frozen in liquid nitrogen and stored at –80°C for lipid analysis and assay of peroxisomal β-oxidation. The other part of homogenates were centrifuged at 18000 x *g* for 20 min, the supernatant was recentrifuged under the same conditions. The resulting supernatant was centrifuged at 105000 x *g* for 60 min. The pellet was resuspended in 0.25 M sucrose /0.1 mM EDTA/10 mM Tris-HCl (pH 7.4) and recentrifuged under the same condition. The resulting pellet (microsomes) was resuspended in a small volume of 0.25 M sucrose/0.1 mM EDTA/10 mM Tris-HCl (pH 7.4) and used as an enzyme source. All the operations mentioned above were carried out at 0-4°C. Protein concentrations were determined by the method of Lowry et al. (19) using BSA as a standard.

Enzyme assays Acyl-CoA oxidase was assayed by measuring palmitoyl-CoA dependent H_2O_2 production according to the method of Small et al. (20), employing homogenates as an enzyme source. Briefly, 1 mL of the reaction mixture contained 50 nmol leuco-dichlorofluorescein, 0.2 mg horse radish peroxidase, 4 μmol aminotriazole, 50 nmol palmitoyl-CoA, 0.02% Triton X-100, 20 mM potassium phosphate buffer (pH 7.4) and 20 μg protein of liver homogenates. After preincubation in the absence of palmitoyl-CoA at 30°C, the reaction was started by adding 50 nmol palmitoyl-CoA and the change in the absorbance at 502 nm was monitored. The activity was calculated assuming absorption coefficient for leuco-dichlorofluorescein is 1.42×10^5 $M^{-1}cm^{-1}$.

SCD activity was assayed spectrophotometrically by the method of Oshino et al. (21) as the stearoyl-CoA stimulated re-oxidation of NADH-reduced cytochrome b_5. The

rate of cytochrome b_5 oxidation was measured by recording the changes in absorbance between 424 and 409 nm at 30 °C. The initial incubation mixture contains 1.2 mg microsomal protein and 100 mM Tris-HCl (pH 7.4). Cytochrome b_5 was reduced by adding 2 nmol NADH and the re-oxidation was recorded. When the re-oxidation was completed, 20 nmol stearoyl-CoA was added, and cytochrome b_5 was reduced again by 2 nmol of NADH. The first order rate constant for the re-oxidation of NADH-reduced cytochrome b_5 was calculated as described by Oshino and Sato (22). The rate constant for the re-oxidation of cytochrome b_5 was measured in the presence (k) and in the absence (k^-) of stearoyl-CoA; the rate constant for SCD was given by $k^+=k-k^-$ (11, 23). Microsomal activities of NADH-cytochrome *c* reductase, NADH-ferricyanide reductase, and the content of cytochrome b_5 were determined as described previously (24).

Fatty acid chain elongation was assayed as previously described (14). Incubation mixture contained 15 nmol palmitoyl-CoA, 100 nmol [2-^{14}C]malonyl-CoA, 0.5 μmol NADH, 0.5 μmol NADPH, 0.5 μmol KCN and 250-500 μg microsomal protein in 0.5 ml of 100 mM Tris-HCl buffer (pH 7.4). The mixture was incubated at 37 °C for 4 min under nitrogen. The incubation mixture without palmitoyl-CoA was run simultaneously. After stopping the enzymatic reaction by the addition of 1 mL of 10% KOH/90% methanol, the mixture was heated at 80 °C for 30 min under nitrogen and then acidified by adding 2 mL of 6 M HCl. Fatty acids were extracted with 3 mL of *n*-hexane four times. The combined *n*-hexane extract was washed with 4 mL of acidic water, transferred to a counting vial, and taken to dryness. The remaining fatty acids were dissolved in toluene scintillator and the radioactivity was measured by a liquid scintillation counter.

1-Acylglycerophosphocholine (1-acly-GPC) acyltransferase was assayed

essentially according to Lands and Heart (25). The reaction mixture contained 20-30 nmol oleoyl-CoA, 150 nmol 1-acyl-GPC, 1 μmol DTNB, and 50-75 μg microsomal protein in a final volume of 1 ml of 100 mM Tris-HCl buffer (pH 7.4). After preincubation in the absence of oleoyl-CoA at 30 °C, the incubation was initiated by adding oleoyl-CoA and the increase in the absorbance at 412 nm was monitored. The value determined in the absence of 1-acyl-GPC was subtracted as a background to provide a net acyl transfer rate.

Acyl-CoA synthetase was assayed using [^{14}C]palmitic acid as a substrate according to Tanaka, et al. (26).

Assay for blood glucose and insulin

Serum levels of glucose and insulin were determined using Glucose-test Wako (Wako Pure Chemicals Inc, Osaka, Japan) and Rat insulin ELISA kit (Mercodia, Uppsala, Sweden), respectively.

Lipid analyses Total lipids were extracted from liver homogenates by the method of Bligh and Dyer (27). For quantification of fatty acids, a known amount of triheptadecanoin was added to the homogenates as an internal standard. After solvent was evaporated, total lipids were added with 1 mL 10% KOH/90% methanol and then heated at 80 °C for 60 min for saponification. Non-saponified lipids were removed by the extraction three times with 3 ml of hexane. After the addition of 1 mL 6M HCl, free fatty acids were extracted three times with 3 ml of hexane. The extract was taken to dryness, and to the residue was added methanolic BF_3 and heated at 100 °C for 10 min. Fatty acid methyl esters formed were extracted with hexane and subjected to GLC

analysis (Simadzu GC-14A, equipped with a flammable ionization detector and Supelcowax 10 (0.32 mm ID x 30 m)).

Statistics Analysis of variance was used to test the significance of differences between control rats, glucose- or fructose-administered rats. Where differences were significant, the statistical significance between any two means were determined using Shèffe's multiple range test. Statistical significance between clofibric acid-treated and untreated rats was analyzed by Student's *t*-test or Welch's test after *F*-test for two means.

RESULTS

Induction of PCE and SCD by glucose and fructose

Administration of glucose caused a significant increase in PCE activity in rat liver. It was required at least four days to reach the maximum activity (Fig. 1). Fructose induced PCE as was observed with glucose. There was no significant difference in the potency to induce PCE between glucose and fructose. Next, we examined whether clofibric acid, a known drug to induce PCE (11), further increase PCE activity in rats administered glucose or fructose. Clofibric acid treatment caused a significant increase in PCE activity 1.9, 1.8 and 1.4 times, respectively, over untreated control in normal, glucose-, and fructose-administered rats (Fig. 2A). The administration of glucose and fructose increased hepatic terminal desaturase activity in SCD system 4.2 and 4.8 times, respectively (Fig. 2B). Nevertheless, clofibric acid did not cause an additional increase in terminal desaturase activity in glucose- or fructose-administered rats (Fig. 2B). In other components of SCD system, NADH-ferricyanide reductase activity and the content of cytochrome b_5 were not altered by carbohydrate administration or clofibric acid treatment while NADH-cytochrome *c* reductase activity was reduced by clofibric acid in both normal and carbohydrate-administered rats (data not shown).

Clofibric acid is known as a peroxisome proliferator and causes the induction of both acyl-CoA oxidase and acyl-CoA synthetase via peroxisome proliferator-activated receptor α (PPARα) activation (28). The drug also induces 1-acyl-GPC acyltransferase; the induction was positively correlated to peroxisomal β-oxidation or acyl-CoA oxidase activity (29). Glucose or fructose administration did not significantly affect the activities of these enzymes (Table 1). In addition, clofibric acid-induced activities of these three enzymes were not different between control, glucose- and fructose-administered rats

(Table 1).

In streptozotocin-induced diabetic rats, the activities of both PCE and SCD were reduced by 70 % (Fig. 3). In diabetic rats, fructose feeding increased the activities of both PCE and SCD, while glucose feeding did not change the activities (Fig. 3).

Effects of clofibric acid on serum levels of insulin and glucose Serum levels of glucose were not significantly different between control, glucose- and fructose-administered normal rats (153.2 ± 12.0, 173.7 ±14.0 and 144.9 ± 3.5 mg/dL, respectively). Clofibric acid treatment did not altered serum glucose level in control and glucose-administered rats (146.4 ± 5.5 and 169.5 ± 6.8 mg/dL, respectively), and slightly increased it in fructose-administered rats (152.3 ± 4.2 mg/dL). In diabetic groups, serum levels of glucose in control, glucose- and fructose-fed rats were 497.2 ± 22.9, 657.5 ± 97.5 and 750.6 ± 145.9 mg/dL, respectively. There was no significant difference in serum insulin level between six experimental groups (average value of all rats was 0.785 + 0.456 ng/mL). In all diabetic rats, serum insulin level was below the detection limit (0.07 ng/mL).

Effects of glucose and fructose administration and clofibric acid treatment on the composition of hepatic fatty acid Fig. 4 shows effects of carbohydrate administration in combination with clofibric acid treatment on the proportion of 18:1(n-9) in liver lipids. The amounts of total fatty acids were not different between six experimental groups. The proportion of 18:1(n-9) in hepatic lipids of clofibric acid-treated rats was 1.7 times higher than that in untreated rats. Glucose and fructose administration caused 1.9- and 1.8-times increase in 18:1(n-9), respectively (Fig. 4).

Treatment with clofibric acid of glucose- and fructose-administered rats further increased 18:1(n-9) 2.1 and 1.6 times, respectively (Fig. 4).

To estimate relative contribution of SCD and PCE to 18:1(n-9) formation, linear regression analyses were performed on the proportion of 18:1(n-9) vs. the activity of PCE or SCD in the liver (Fig. 5). There was a highly significant correlation between 18:1(n-9) proportion and PCE activity (r^2=0.8743, P<0.01), while the correlation between the proportion of 18:1(n-9) and SCD activity was not statistically significant (r^2=0.5521, P>0.05).

DISCUSSION

Induction of PCE by glucose and fructose

In the present study, we demonstrated that both glucose and fructose induced PCE in the liver of rats as well as SCD (5). The effects of the carbohydrates were not mediated by insulin, a known inducer of both PCE and SCD, because serum insulin level was not altered by the administration of carbohydrates in normal and diabetic rats. Differed from normal rats, only fructose but not glucose could increase the activities of PCE and SCD in diabetic rats (Fig. 3). Although the mechanism by which fructose induces PCE and SCD in diabetic animals has not yet clarified. It is plausible that intermediates of glycolytic pathway plays a pivotal role because fructokinase is not affected while glucokinase was greatly depressed in diabetic state (30).

Comparison of the induction between SCD and PCE The early studies showed that PCE activity concomitantly increased with an increase in SCD activity by administration of clofibric acid (11, 24, 31), diethylhexylphthalate (10), re-feeding of fat-free diet after fasting and treatment of diabetic rats with insulin (10, 11), and, on the contrary, diabetic state and starvation reduced the activities of both SCD and PCE (5, 10, 15). These findings suggest that both PCE and SCD are regulated by the similar mechanisms. Recent studies have revealed that SCD is trasncriptionally regulated by insulin, carbohydrate and polyunsaturated fatty acids via sterol regulatory element-binding protein (7-9, 32). In contrast to SCD, little attention has been paid to the regulation of PCE except for the early studies on the changes in the activity in various physiological conditions (10, 11, 15). More recent studies showed the possibility that the mRNA expression of fatty acid chain elongase is regulated by sterol regulatory

element binding protein-1 (33, 34). Therefore, there is a possibility that the induction of PCE by carbohydrates is mediated by sterol regulatory element binding protein. Induction of PCE and SCD by carbohydrate was independent of PPARα because carbohydrate did not induce acyl-CoA synthetase, acyl-CoA oxidase and 1-acyl-GPC acyltransferase and did not modify the induction of them with clofibric acid (Table 1).

Both PCE and SCD are known to be induced by peroxisome proliferators, such as clofibric acid and diethylhexylphthalate (10, 11, 24, 31). Peroxisome proliferators induce various enzymes and proteins that are responsible for fatty acid transport and oxidation by transcriptional activation via PPARα binding to peroxisome proliferator responsive element in their promoter region (27, 35). Treatment of mice with clofibrate (ethyl ester of clofibric acid) increased mRNA level of SCD only 2-fold (36), which was far less than the increase in mRNA levels of fatty acid oxidizing enzymes (28). It is, therefore, unclear whether clofibric acid induces PCE and SCD by direct activation of PPARα although Miller et al. found PPRE-like sequence in the promoter region of mouse SCD1 (37). We observed that clofibric acid did not further increase SCD activity in carbohydrate-administered rats (Fig. 2). It is plausible that clofibric acid indirectly induces SCD by altering fatty acid metabolism. Even though the induction by clofibric acid is dependent on PPARα, physiological significance seems to be low compared to the regulation by carbohydrate. By contrast to SCD, PCE was additively induced by clofibric acid and carbohydrate. The question whether PPAR directly promote PCE expression remains to be elucidated.

Role of PCE in 18:1(n-9) formation The similarity in the regulation of PCE and SCD raised a question of which enzyme is more important in the regulation of oleic acid

formation. Our present study demonstrated a difference in the induction between SCD and PCE upon clofibric acid treatment in combination with carbohydrate. Namely, clofibric acid treatment additionally increased PCE activity in carbohydrate-treated rats, whereas no additional increase with clofibric acid treatment was observed in SCD activity (Figs. 2 and 3). These results imply that SCD and PCE differently contribute to oleic acid formation in response to various stimuli. In fact, hepatic content of oleic acid was significantly increased where only PCE activity was increased by the treatment of carbohydrate-administered rats with clofibric acid (Table 3). Highly significant correlation between the proportion of oleic acid and PCE activity (r^2=0.8743, P<0.01), but not SCD activity (r^2=0.5521, P>0.05) clearly indicates physiological importance of PCE in oleic acid formation. To our knowledge, this is the first report that demonstrated the importance of PCE in comparison with SCD in oleic acid formation.

The resent study indicates that carbohydrate can induce not only SCD but also PCE and that PCE plays a crucial role in the changes in the formation of 18:1(n-9).

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REFERENCES

1. Cinti, D.L., Cook, L., Nagi, M.N. and Suneja, S.K. (1992) The Fatty Acid Chain Elongation System of Mammalian Endoplasmic Reticulum, *Prog. Lipid Res.* 31, 1-51.
2. Jeffcoat, R. (1979) The Biosynthesis of Unsaturated Fatty Acids and Its Control in Mammalian Liver, *Essays Biochem.* 15, 1-36.
3. Thiede, M.A., Ozol, J. and Strittmatter, P. (1986) Construction and Sequence of cDNA for Rat Liver Stearyl Coenzyme A Desaturase, *J. Biol. Chem.* 261, 13230-13235.
4. Ohsino, N. and Sato, R. (1972) The Dietary Control of he Microsomal Stearyl CoA Desaturation Enzyme System in Rat Liver, *Arch. Biochem. Biophys.* 149, 369-377.
5. Prasad, M.R. and Joshi, V.C. (1979) Regulation of Rat Hepatic Stearoyl Coenzyme A Desaturation. The Roles of Insulin and Carbohydrate, *J. Biol. Chem.* 254, 997-999.
6. Jeffcoat, R. and James, A.T. (1978) The Control of Stearoyl-CoA Desaturase by Dietary Linoleic Acid, *FEBS Lett.* 85, 114-118.
7. Shimano, H., Yahagi, N., Amemiya-Kudo, M., Hasty, A., Osuga, J., Tamura, Y., Shiomori, F., Iizaka, Y., Ohashi, K., Harada, K., Gotoda, T., Ishibashi, S. and Yamada, N. (1999) Sterol Regulatory Elenent-Binding Protein-1 as a Key Transcription Factor for Nutritional Induction of Lipogenic Enzyme Genes, *J. Biol. Chem.* 274, 35832-35839.
8. Horton, J.D., Bashmakov, Y., Shimomura, I. And Shimano, H. (1998) Regulation of Sterol Regulatory Element-Binding Proteins in Livers of Fasted and Refed Mice,

Proc. Natl. Acad. Sci. USA 95, 5987-5992.

9. Xu, J., Nakamura, M.T., Cho, H.P. and Clarke, S.D. (1999) Sterol Regulatory Element-Binding Protein-1 Expression is Suppressed by Dietary Polyunsaturated Fatty Acids. A Mechanism for the Coodinate Suppression of Lipogenic Genes by Polyunsaturated Fats, *J. Biol. Chem.* 274, 23577-23583.
10. Prasad, M.R., Nagi, M.N., Ghesquier, D., Cook, L. and Cinti, D.L. (1986) Evidence for Multiple Condensing Enzymes in Rat Hepatic Microsomes Catalyzing the Condensation of Saturated, Monounsaturated, and Polyunsaturated Acyl Coenzyme A, *J. Biol. Chem.* 261, 8213-8217.
11. Kawashima, Y. and Kozuka, H. (1985) Regulation of Palmitoyl-CoA Chain Elongation and Linoleoyl-CoA Chain Elongation on Rat Liver Microsomes and the Different Effects of Peroxisome Proliferators, Insulin and Thyroid Hormone, *Biochim. Biophys. Acta*, 834, 118-123.
12. Luthria, D.L. and Sprecher, H. (1997) Studies to Determine if Rat Liver Contains Multiple Chain Elongating Enzyme, *Biochim. Biophys. Acta* 1346, 221-230.
13. Alegrel, M., Cerqueda, E., Vazquez, M., Sanchez, R.M., Adzet, T., Merlos, M. and Laguna, J.C. (1991) Selective Modification of Rat Hepatic Microsomal Fatty Acid Chain Elongation and Desaturation by Fibrates: Relationshipwith Peroxisome Proliferation, *Br. J. Pharmacol.* 114, 1351-1358.
14. Kawashima, Y., Suzuki, Y. and Hashimoto, Y. (1977) Dietary Control of the Chain Elongation of Palmitoyl-CoA in Rat Liver Microsomes, *Lipids* 12, 434-437.
15. Suneja, S.K., Osei, P., Cook, L., Nagi, M.N. and Cinti, D.L. (1990) Enzyme Site-specific Changes in Hepatic Microsomal Fatty Acid Chain Elongation in Streptozotocin-Induced Diabetic Rats, *Biochim. Biophys. Acta*, 1042, 81-85.

16. Uchiyama, M. and Kawashima, Y. (1977) Age Related Alterations in Chain Elongation and Monounsaturation of Fatty Acyl-CoA in Rat Liver Microsomes, *Exp. Geront.* 13, 57-61.

17. Laguna, J.C., Nagi, M.N., Cook, L. and Cinti, D.L. (1989) Action of Ebselen on Rat Hepatic Microsomal Enzyme-Catalyzed Fatty Acid Chain Elongation, Desaturation, and Drug Biotransformation, *Arch. Biochem. Biophys*, 269, 272-283.

18. Sanchez, R.M., Vinals, M., Alegret, M., Vazquez, M., Adzet, T, Merlos, M. and Laguna, J.C. (1993) Fibrates Modifiy Rat Hepatic Fatty Acid Chain Elongation and Desaturation *in vitro*, *Biochem. Pharmacol.* 46, 1791-1796.

19. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) Protein Measurement with the Folin Phenol Reagent, *J. Biol. Chem.* 193, 265-275.

20. Small, G.M., Burdett, K. and Cnnock, M. (1985) A Sensitive Spectrophotometric Assay for Peroxisomal Acyl-CoA Oxidase, *Biochem. J.* 227, 205-210.

21. Oshino, N., Imai, Y. and Sato, R. (1971) A Function of Cytochrome *b5* in Fatty Acid Desaturation by Rat Liver Microsomes, *J. Biochem. Tokyo* 69, 155-167.

22. Oshino, N. and Sato, R. (1971) Stimulation by Phenol of the Reoxidation of Microsomal Bound Cytochrome b_5 and its Implication to Fatty Acid Desaturation, *J. Biochem. Tokyo*, 69, 169-180.

23. Hoch, F.L., DePierre, J.W. and Ernster, L. (1980) Thyroid Control over Biomembranes. Liver-Microsomal Cytochrome b_5 in Hypothyroism. *Eur. J. Biochem.* 109, 301-306.

24. Kawashima, Y., Hanioka, N., Matsumura, M. and Kozuka, H. (1983) Induction of Microsomal Stearoyl-CoA Desaturation by the Admnisitration of Various Peroxisome Proliferators, *Biochim. Biophys. Acta*, 752, 259-264.

25. Lands, W.E.M., and Heart, P. (1965) Metabolism of Glycerolipids. VI. Specificities of Acyl esters of Coenzyme A: Phospholipid Acyltransferases, *J. Biol. Chem.* 240, 1905-1911.

26. Tanaka, T., Hosaka, K., Hoshimaru, M. and Numa, S. (1979) Purification and Properties of Long Chain Acyl-Coenzyme A Synthetase from Rat Liver, *Eur. J. Biochem.* 98, 165-172.

27. Bligh, E.G. and Dyer, W.J. (1959) A Rapid Method of Total Lipid Extraction and Purification, *Can. J. Biochem. Physiol.* 37, 911-917.

28. Schoonjans, K., Staels, B. and Auwerx, J. (1996) Role of Peroxisome Proliferator-Activatd Receptor (PPAR) in Mediating the Effects of Fibrates and Fatty acids on Gene Expression, *J. Lipid Res.* 37, 907-325.

29. Kawashima, Y., Horii, S., Matsunaga, T., Hirose, A., Adachi, T. and Kozuka, H. (1989) Co-Induction by Peroxisome Proliferators of Microsomal 1-Acylglycerophosphocholine Acyltransferase with Peroxisomal β-Oxidation in Rat Liver, *Biochim. Biophys. Acta* 1005, 123-129.

30. Adelman, R.C., Spolter, P.D., Weinhouse, S., (1966) Dietary and Hormonal Regulation of Enzymes of Fructose Metabolism in Rat Liver, *J. Biol. Chem.* 241, 5467-5472.

31. Kawashima, Y., Hirose, A., Kozuka, H. (1984) Modification by Clofibric acid of Acyl Compoaition of Glycerolipids in Rat Liver. A Possible Involvement of Fatty Acid Chain Elongation and Desaturation, *Biochim. Biophys. Acta* 795, 543-551.

32. Tabor, D.E., Kim, J. B., Spiegelman, B.M. and Edwards, P.A. (1998) Transcriptional Activation of the Stearoyl-CoA Desaturase Gene by Sterol Regulatory Element-Binding Protein/Adipose Determination and Differentiation

Factor A, *J. Biol. Chem.* 273, 22052-22058.

33. Moon, Y.-A., Shah, N.A., Mohapatra, S., Warrington, J. and Horton, J.D. (2001) Identification of a mammalian Long Chain Fatty Acyl Egongase Regulated by Sterol Regulatory Element-Binding Proteins, *J. Biol. Chem.* 276, 45358-45366.

34. Matsuzaka, T., Shimano, H., Yahagi, N., Yoshikawa, T., Amemiya-Kudo, M., Hasty, A., Okazaki, H., Tamura, Y., Iizuka, Y., Ohashi, K., Osuga, J, Takahashi, A., Yato, S., Sone,H., Ishibashi, S. and Yamada, N. (2002) Cloning and Characterization of a Mammalian Fatty Acyl-CoA Elongase as a Lipogenic Enzyme Regulated by SREBPs. *J. Lipid Res.* 43, 911-920.

35. Motojima, K., Passilli, P., Peters, J.M., Gonzales, F.J. and Latruffe, N. (1998) Expression of Putative Fatty Acid Transporter Genes are regulated by Peroxisome Proliferator-Activated Receptor a and g activators in a Tissue- and Inducer-Specific Manner, *J. Biol. Chem.* 273, 16710-16714.

36. Diczfalusy, U., Eggertsen, G. and Alexson, S.E.H. (1995) Clofibrate Treatment Increases Stearoyl-CoA Desaturase mRNA Level and Enzyme Activity in Mouse Liver, *Biochim. Biophys. Acta*, 1259, 313-316.

37. Miller, C.W. and Ntambi, J.M. (1996) Peroxisome Proliferators Induce Mouse Liver Stearoyl-CoA Desaturase 1 Gene Expression, *Proc. Natl. Acad. Sci. USA.* 93, 9443-9448.

TABLE 1 Effects of Glucose, Fructose and Clofibric acid on the Activites of Acyl-CoA Synthetase, Acyl-CoA Oxidase and 1-Acyl-GPC Acyltransferase in the Liver of Rats

	Acyl-CoA synthetase	Acyl-CoA oxidase	1-Acyl-GPC acyltransferase
	(nmol/min/mg protein)	(nmol/min/mg protein)	(nmol/min/mg protein)
Control	73.9 ± 6.2 [ab]	4.58 ± 0.28 [a]	96.3 ± 9.4
Control + clofibric acid	143.8 ± 7.5 *	31.78 ± 3.98 *	184.2 ± 12.4 *
Glucose	87.5 ± 9.5 [b]	4.53 ± 0.66 [a]	100.0 ± 8.4
Glucose + clofibric acid	147.5 ± 11.3 *	31.10 ± 2.10 *	188.6 ± 11.5 *
Fructose	65.5 ± 7.4 [a*]	5.63 ± 0.43 [b]	86.0 ± 7.0
Fructose + clofibric acid	128.8 ± 11.5 *	29.10 ± 0.47 *	197.8 ± 16.9 *

Rats were given 20% wt/vol glucose or fructose solution for drinking for 4 days. Half of them were subcutanously injected with clofibric acid (100 mg/kg body weight) twice a day. Values are means ± SD for 3-5 rats. *, Significantly different from clofibric acid-untreated rats ($P<0.001$). [a,b], Difference between control, glucose-, and fructose-administered rats are statistically significant without a common superscript. If no superscript appears, differences between three groups are not statistically significant.

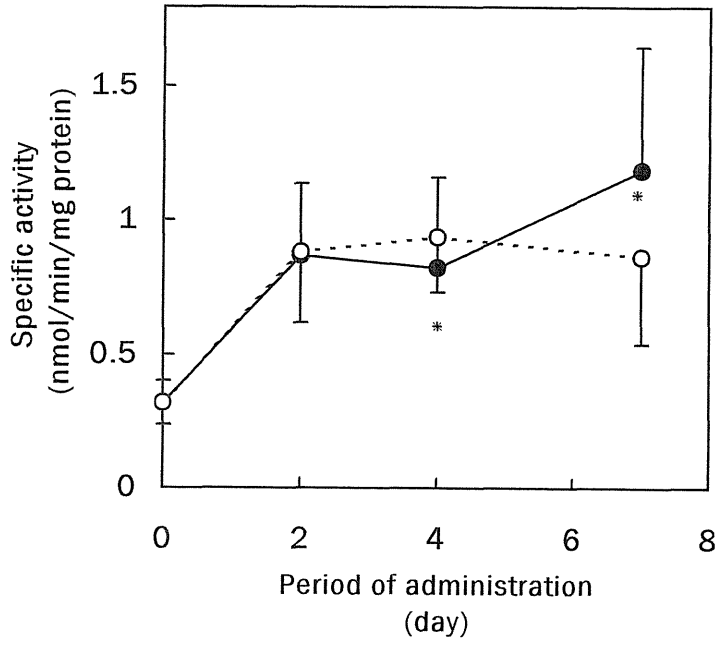


Figure 1 Time course of the induction of hepatic PCE by the administration of glucose and fructose. Liver microsomes were prepared from the rats that were given a 20 % wt/vol solution of glucose (closed circle) or fructose (open circle) for drinking. Values are means ± SD for 3-8 rats. *, Significantly different from control ($P<0.05$).

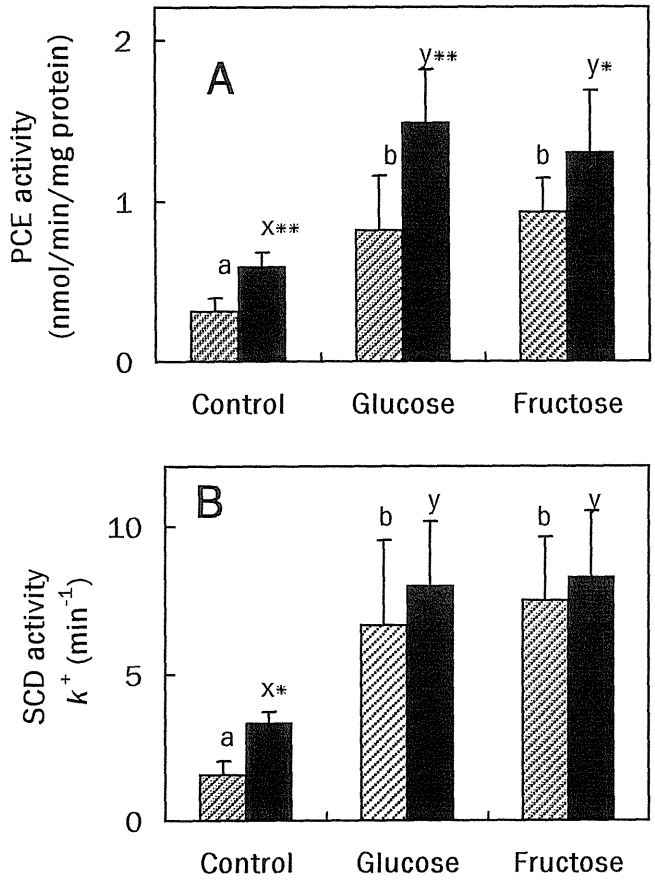
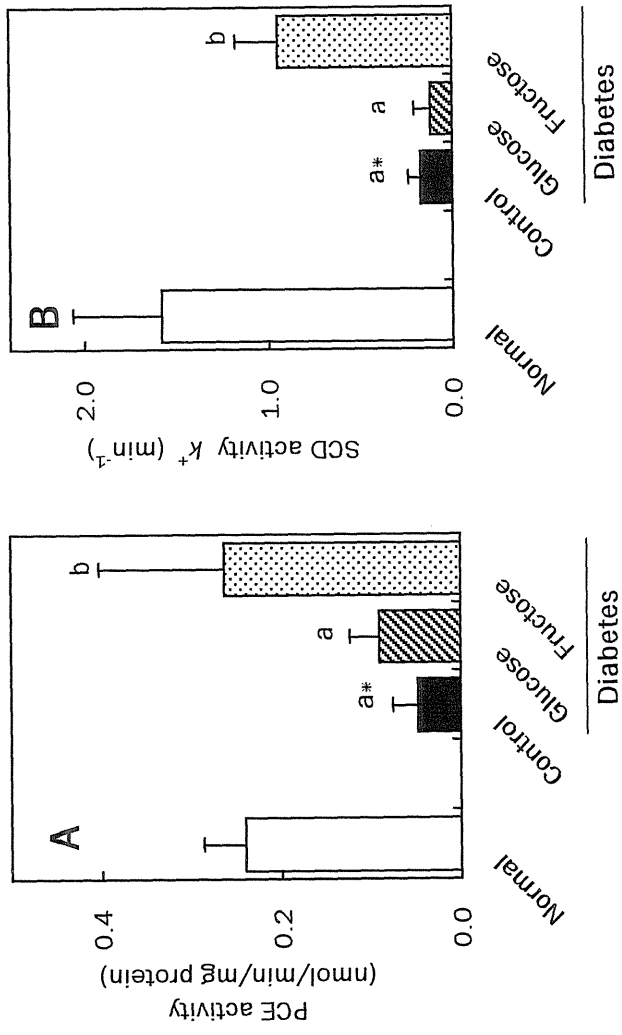


Figure 2 Effects of glucose and fructose in combination with clofibric acid on the activities of PCE and SCD in rat liver. Rats were given a 20% wt/vol solution of glucose or fructose for drinking for four days with (closed bar) or without (hatched bar) subcutaneous injection of clofibric acid twice a day at a dose of 100 mg /kg body weight. Values are means ± SD for 4-8 rats. A, PCE; B, SCD. ^{a,b}, Differences between control, glucose- and fructose-administered rats are statistically significant without a common superscript ($P<0.05$). ^{x,y}, Differences between control, glucose- and fructose-administered rats with clofibric acid treatment are statistically significant without a common superscript ($P<0.05$). *,**, Significantly different from the value of clofibric acid-untreated rats ($P<0.05$ and $P<0.001$, respectively).

Figure 3 Effects of carbohydrate on the activities of PCE and SCD in the liver of streptozotocin-induced diabetic rats. Rats were intravenously injected with streptozotocin (60 mg/kg body weight). Three weeks after the injection, these rats were fed a fat-free diet containing glucose or fructose as a carbohydrate for four days and simultaneously received subcutaneous injection of clofibric acid (100 mg/kg body weight) twice a day. Microsomes were prepared from the rats and the activities of PCE (A) and SCD (B) were determined. *, Significantly different from normal rats ($P<0.05$). [a,b], Differences between control, glucose- and fructose-administered diabetic rats are statistically significant without a common superscript ($P<0.05$).



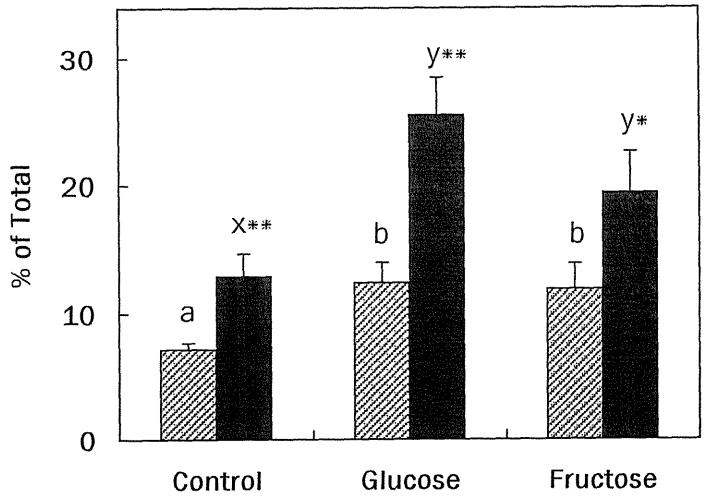


Figure 4 Effects of glucose and fructose in combination with clofibric acid on hepatic proportion of 18:1(n-9). Rats were given a 20% wt/vol solution of glucose or fructose for drinking for four days with (closed bar) or without (hatched bar) subcutaneous injection of clofibric acid twice a day at a dose of 100 mg /kg body weight. Fatty acid composition of total lipids was analyzed in the liver. Values are means ± SD for 3-4 rats. ^{a,b}, Differences between control, glucose- and fructose-administered rats are statistically significant without a common superscript ($P<0.05$). ^{x,y}, Differences between control, glucose- and fructose-administered rats with clofibric acid treatment are statistically significant without a common superscript ($P<0.05$). *,**, Significantly different from the value of clofibric acid-untreated rats ($P<0.05$ and $P<0.01$, respectively).

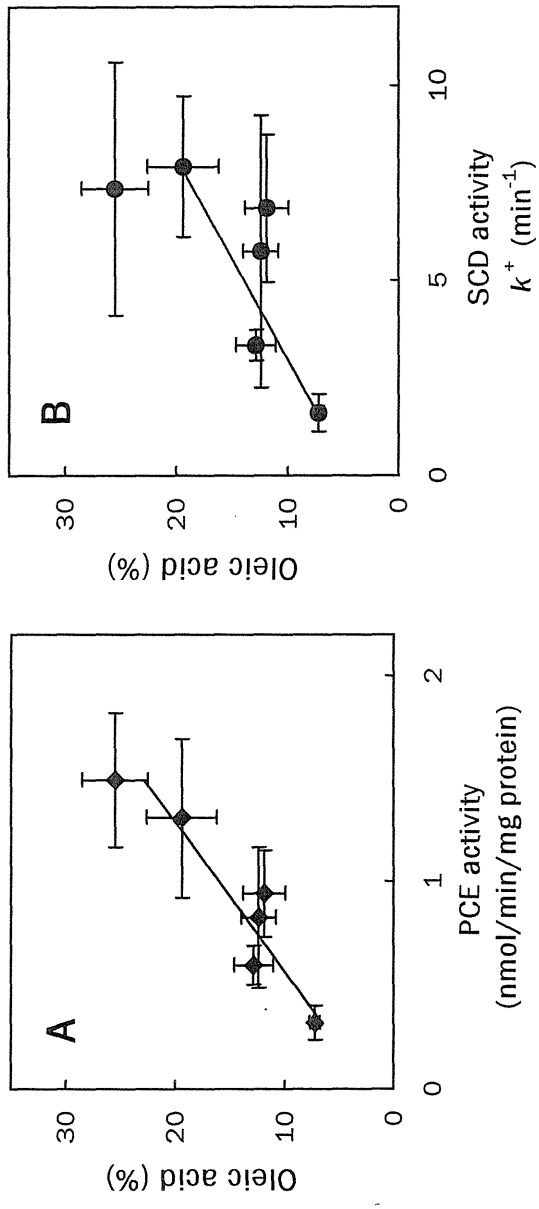


Figure 5 Relationship between the activity of PCE or SCD and proportion of oleic acid in the liver. A, The relationship between the activity of PCE (data from Fig. 2A) and oleic acid proportion (data from Fig. 4) is determined as Y=13.955X + 2.152 (r^2=0.8743, P<0.01). B, The relationship between the activity of SCD (data from Fig. 2B) and oleic acid proportion is calculated as Y=1.934X + 4.294 (r^2=0.5521, P>0.05).